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Expression of *Arabidopsis* CBF1 regulated by an ABA/stress inducible promoter in transgenic tomato confers stress tolerance without affecting yield

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ABSTRACT

Modern-day plants are subjected to various biotic and abiotic stresses thereby limiting plant productivity and quality. It has previously been reported that the use of a strong constitutive 35S cauliflower mosaic virus (CaMV) promoter to drive the expression of *Arabidopsis CBF1* in tomato improved tolerance to cold, drought and salt loading, at the expense of growth and yield under normal growth conditions. Hence in the present study, the suitability of expressing the *Arabidopsis CBF1* driven by three copies of an ABA-responsive complex (ABRC1) from the barley *HAV22* gene in order to improve the agronomic performance of the transgenic tomato plants was investigated. Northern blot analysis indicated that *CBF1* gene expression was induced by chilling, water-deficit and salt treatment in the transgenic tomato plants. Under these tested stress conditions, transgenic tomato plants exhibited enhanced tolerance to chilling, water-deficit, and salt stress in comparison with untransformed plants. Under normal growing conditions the ABRC1-*CBF1* tomato plants maintained normal growth and yield similar to the untransformed plants. The results demonstrate the promise of using ABRC1-*CBF1* tomato plants in highly stressed conditions which will in turn benefit agriculture.

Key-words: abscisic acid; *CBF1*; osmotic stress; transgenic tomato.

Abbreviations: ABA, abscisic acid; ABRC, ABA-response complex; ABREs, ABA-response elements; *CBF1*, C-repeat/dehydration responsive element binding factor 1.

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INTRODUCTION

Abiotic stress in the broadest sense encompasses cold, drought and salt stress. These are the major limiting factors in agricultural productivity and quality, thus preventing crop plants from realizing their full genetic potential. Crops have evolved complex physiological and biochemical sensing and responsive systems to cope with the physical environments. The products of these genes may participate in the generation of regulatory molecules such as plant hormones, abscisic acid (ABA), ethylene and salicylic acid (SA). These regulatory molecules modulate secondary messengers such as Ca^{2+} initiating the protein phosphorylation cascade that finally targets protein directly involved in cellular protection or the transcriptional factor controlling specific sets of stress-regulated genes (Xiong, Schumaker & Zhu 2002).

Several stress-induced *cor* genes such as *rd29A (cor78)*, *cor15A*, *kin1* and *cor6.6* are triggered in response to cold treatment, ABA and water-deficit stress (Thomashow 1998). C-repeat (CRT) and dehydration responsive element (DRE)-related motifs have been reported in the promoter sequences of these genes (Horvath, McLarney & Thomashow 1993; Nordin, Vahala & Palva 1993; Baker, Willhelm & Thomashow 1994; Wang & Cutler 1995). The cDNA encoding the CRT/DRE-binding protein *CBF1* has been isolated by the yeast one-hybrid system (Stockinger, Gilmour & Thomashow 1997). Overexpression of *Arabidopsis CBF1* has been shown to activate *cor* homologous genes at non-acclimating temperatures (Jaglo *et al.* 2001). In addition *Arabidopsis* DRE binding factor genes DREB1A and DREB2A containing the ERBP/AP2 DNA-binding domain (Stockinger *et al.* 1997; Liu *et al.* 1998) operate in two cellular signal transduction pathways, namely in response to low temperature and water deficit, respectively (Liu *et al.* 1998). Results from these studies suggest that these newly identified gene products including *CBF1*, *DREB1A* and *DREB2A* have a bearing in the control of stress response (Jaglo *et al.* 2001).

Genes responsible for tolerance to various abiotic stresses such as those encoding enzymes required for bio-

synthesis of various osmoprotectants, late-embryogenesis-abundant (LEA) proteins and modifying membrane lipids, have been successfully transferred by several gene transfer techniques in model plants (Holmberg & Bulow 1998). Each of these experiments involved transfer of stress-responsive genes to either single or multiple stresses, in model plants such as *Arabidopsis*, tobacco and alfalfa to demonstrate stress tolerance. To investigate the possibility of enhancing tolerance towards multiple stresses (cold, drought and salt) in a practical crop (*Lycopersicon esculentum*), we transferred a cDNA encoding CRT/DRE binding factor 1 (*CBF1*) isolated from *Arabidopsis* under the control of CaMV35S. Overexpression in tomato improved tolerance to chilling, drought and salt stress but resulted in a dwarf phenotype and reduction in fruit set and seed number per fruit (Hsieh *et al.* 2002a, b). Hence we reasoned that the development of transgenic tomato plants with optimized tolerance to abiotic stress and minimal effects on phenotype and yield may necessitate the overexpression of *Arabidopsis CBF1* under the control of a stress-inducible promoter as demonstrated by Kasuga *et al.* (1999) in *Arabidopsis*. Hence, in the present investigation we have used three copies of a stress-inducible ABRC1 promoter from the barley *HAV22* gene to drive the expression of *Arabidopsis CBF1*, with the aim of enhancing tolerance to multiple stresses and minimizing the negative effects in transgenic tomato plants pertaining to growth and yield compared with the use of the CaMV35S promoter.

MATERIALS AND METHODS

Plasmid construction

Arabidopsis thaliana L. Hyen ecotype Colombia were grown in pots under controlled conditions at 22/24 °C, 50% relative humidity and 24 h photoperiod (120 μmol m^{-2} s^{-1}). A *CBF1* gene was isolated by reverse transcriptase (RT)-polymerase chain reaction (PCR) from 3-week-old *Arabidopsis* seedlings as described previously (Hsieh *et al.* 2002a). The ABRC3-*CBF1* was constructed as follows. The *CBF1* gene was cloned into the *Sal*I-*Sac*I site of pJD301, to form the *CBF1*/pJD301 intermediate vector. The ABRC1 promoter (three copies of ABRC1) from the barley *HAV22* gene fused to a truncated (-60) barley alpha amylase (*Am64*) promoter was digested with *Not*I and *Nco*I from pQS122 (Su *et al.* 1998). This was filled by Klenow fragment and ligated to the *Hin*dIII and *Sal*I site of *CBF1*/pJD301. The ABRC1/*CBF1*/nos was cloned into the *Eco*RI site of pCAMBIA 2301 and designated as pJLM2. The construct was introduced into *Agrobacterium tumefaciens strain* LBA4404 by electroporation.

Plant transformation

Tomato seeds (*Lycopersicon esculentum* Mill. cv CL5915–93D4-1-013) was kindly provided by the Asian Vegetable Research and Development Center. The seeds were surface sterilized with 1% bleach for 10 min and finally washed with sterilized distilled water three to four times. Twenty seeds were sown in bottles containing 50 mL Murashige and Skoog (MS) basal medium and incubated at 26/24 °C (day/night) under a 16/8 h (day/night) photoperiod. Light was provided by cool-white fluorescent lamps at photosynthetic photon flux of 120 μmol m^{-2} s^{-1}. Cotyledon explants of 8- to 10-day-old-seedlings were excised and transformed as mentioned previously (Hsieh *et al.* 2002b). The transgenic tomato plants selected on kanamycin medium were grown in 9 cm pots containing a 1 : 1 mixture of perlite and vermiculite under controlled conditions at 26/24 °C (day/night) under a 16/8 h (day/night) photoperiod. Relative humidity was maintained at 50%. The plants were irrigated with Hoagland solution every 6 d.

Stress treatment of transgenic tomato plants

Transgenic T_1 (AC1, AC2 and AC3) and untransformed tomato plants were grown in pots (60 cm × 20 cm × 15 cm) containing a mixture of perlite and vermiculite (1 : 1) with a 16/8 h (day/night) photoperiod (about 120 μmol m^{-2} s^{-1}) at 26/24 °C (day/night). Two-month-old plants were exposed to chilling (20 replicates), drought (30 replicates) and salt stress (30 replicates). Freezing stress was conducted by exposing plants to 0 °C for 7 d. Drought stress was conducted by withholding water for 4 weeks. Irrigating the plants with 200 mM NaCl for 4 weeks created high salt stress. All the tomato plants were restored to normal growth conditions after their respective treatments. The tomato plants that survived the stress treatments were counted and divided by the total plant number to define the survival rate. Pictures were taken to record the phenotypes.

Molecular analysis of ABRC1-*CBF1* tomato plants

Leaves from 2-month-old T_2 tomato plants subjected to various stress treatments as described above were collected and frozen in liquid nitrogen for further analysis. Total DNA as well as RNA for Southern and Northern blot analysis were isolated from leaves of transgenic and untransformed tomato plants as described previously (Hsieh *et al.* 2002b). The *CBF1* and *CAT1* genes isolated from pT7 Blue® (Novagen, Madison, WI) were labelled with (α-^{32}P) dCTP by the random primer method and used as probes.

Reversibility of *CBF1* expression

Transgenic tomato plants were evaluated for the reversibility of the *CBF1* expression. Two-month-old T_2 transgenic (AC2) and untransformed tomato plants were grown in pots (60 cm × 20 cm × 15 cm) containing a mixture of perlite and vermiculite (1 : 1) with a 16/8 h (day/night) photoperiod (about 120 μmol m^{-2} s^{-1}) at 26/24 °C (day/night). These plants were subjected to chilling, drought and salt treatment as follows. Chilling treatment was conducted by exposing the tomato plants to 0 °C for 2 d followed by

restoring normal growth conditions for 4 d. Drought stress was conducted by withholding water for 3 d followed by watering for 4 d. Similarly salt stress was conducted by growing the tomato plants in 200 mM NaCl for 3 d followed by restoring normal growth conditions for 4 d. Chilling, drought and salt stress treatments were repeated again before restoring the plants to normal growth conditions. Leaf samples were collected during every stage and frozen in liquid nitrogen. Total RNA was extracted as mentioned previously and the blots were probed with *CBF1* labelled with (α-^{32}P) dCTP.

Physiological changes in ABRC1-*CBF1* transgenic tomato plants

Two-month-old T_2 transgenic (AC1, AC2 and AC3) and untransformed tomato plants growing in pots (60 cm × 20 cm × 15 cm) containing a mixture of perlite and vermiculite (1 : 1) with a 16/8 h (day/night) photoperiod (about 120 μmol m^{-2} s^{-1}) at 26/24 °C (day/night) were analysed for the physiological changes such as leaf conductance, ion leakage and chlorophyll fluorescence. Five replicates of transgenic (AC1, AC2 and AC3) and untransformed tomato plants were used for each parameter separately. A similar set of plants growing under normal conditions served as control.

For leaf conductance measurement the plants were subjected to chilling for 2 d, drought treatment was conducted by withholding water for 10 d and salt stress was created by growing the plants in 200 mM NaCl for 4 d. Leaf conductance was then measured using the third and fourth leaves of non-stressed and stressed plants during the treatment phase at different durations (0, 2, 4, 6, 8, 10 and 12 h) with a Li-Cor LI-1600 steady state porometer (Li-Cor Inc., Lincoln, NE, USA) by the method previously described (Hsieh *et al.* 2002a).

Similarly the third and fourth leaves of non-stressed and stressed transgenic and untransformed plants were used for analysis of chlorophyll fluorescence and ion leakage after the respective stress treatments. Chlorophyll fluorescence was analysed with a pulse-activated modulation fluorimeter (Walz, Effeltrich, Germany) according to the method described by Obershall *et al.* 2000). Similarly leaf samples were excised and immersed in deionized water for ion leakage measurement. The ion leakage was determined with a conductivity meter. The samples were autoclaved to destroy the cells and release all ions. The value obtained after autoclaving was designated as 100% leakage.

Analysis of growth performance of ABRC1-*CBF1* tomato plants

The agronomic performance of transgenic (AC1, AC2 and AC3) and untransformed tomato plants were evaluated with respect to fruit number, seed per fruit and fresh weight. Two-month-old T_2 transgenic (AC1, AC2 and AC3) and untransformed plants were grown in pots (60 cm × 20 cm × 15 cm) containing a mixture of perlite and vermiculite (1 : 1) with 16/8 h (day/night) photoperiod (about 120 μmol m^{-2} s^{-1}) at 26/24 °C (day/night). Chilling stress was conducted by exposing the plants to 0 °C for 4 d. Drought stress was conducted by with holding water for 2 weeks. Salt stress was created by growing the plants in 200 mM NaCl for 2 weeks. After the stress treatments the tomato plants were restored to normal growth conditions. Similarly another set of plants subjected to normal growth conditions served as control. The experimental design contained five individual lines (untransformed and transformed) for each treatment. Three-month-old plants were harvested and the fresh weight, fruit number and seed number per fruit were calculated.

RESULTS

Tolerance of ABRC1-*CBF1* transgenic plants to cold, drought and salt stress

About 50 kanamycin-resistant tomato plants were regenerated successfully from tomato cotyledon explants transformed with LBA4404/pJLM2 and transferred to pots. The transgenic plants were confirmed by Southern blot hybridization (unpublished results). Three T_2 transgenic tomato plants were selected randomly and designated as AC1, AC2 and AC3. Untransformed and transgenic (AC1, AC2 and AC3) tomato plants were subjected to drought, salt (30 replications) and chilling stress (20 replications). Figure 1a–c clearly depicts the effects of stress treatment on growth. Chilling treatment was conducted at 0 °C for 7 d (Fig. 1a), withholding water for 4 weeks and irrigating the plants with 200 mM NaCl created drought (Fig. 1b) and salt (Fig. 1c) stress, respectively. In all instances the severity of the damage caused by the stress treatment was considerably less in the transgenic plants (AC1, AC2 and AC3) in comparison with the untransformed plants except in the case of chilling stress in which the difference was not noticeable. When the stress-treated tomato plants were restored to normal growth conditions the survival rate was higher in the transgenic lines subjected to chilling (65, 50 and 55%) drought (83.3, 93.3 and 96.7%) and salt (83.3, 93.3 and 96.7%) stress as compared to the untransformed plants.

Molecular analysis of ABRC1-*CBF1* plants

The expression of ABRC1-*CBF1* was analysed both under non-stress and stress conditions. Under non-stressed condition the untransformed and transgenic lines (AC1, AC2 and AC3) showed no expression of *CBF1* but a low expression of the *CAT1* gene was observed (Fig. 2a) under this condition. Under osmotic stress (drought and salt) treatment ABRC1-*CBF1* plants showed a strong expression of *CBF1* and *CAT1* genes (Fig. 2b & c). In the transgenic lines exposed to chilling treatment the *CBF1* expression was noticeably lower but there was a strong expression of the *CAT1* gene.

The transgenic plants (AC2) were examined for the reversibility of ABRC1-*CBF1* expression. From Fig. 3a it

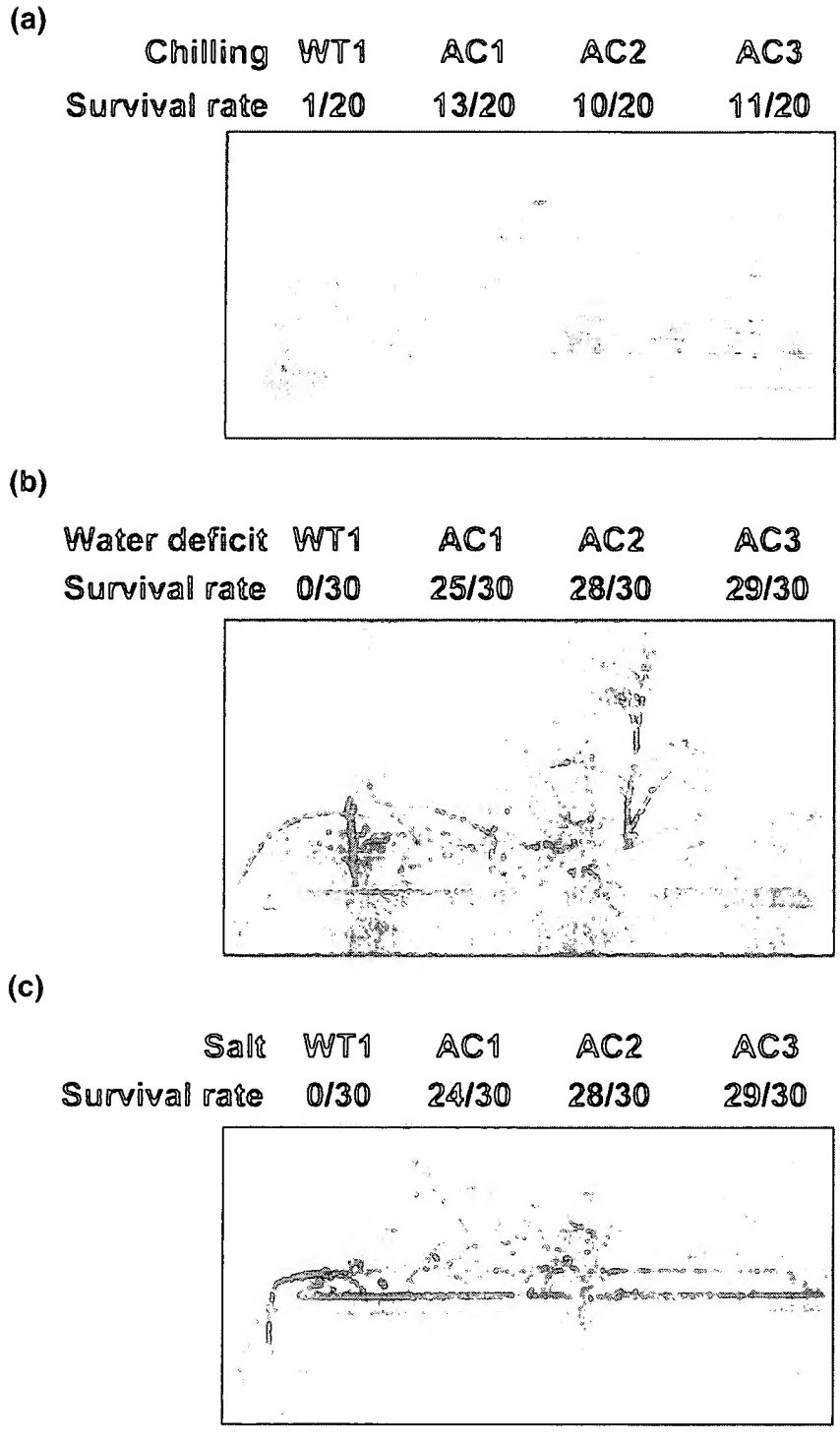

Figure 1. Transgenic ABRC1-*CBF1* tomato plants exhibited more tolerance to chilling and osmotic stresses than untransformed plants. Untransformed and transgenic T_1 plants (WT, AC1, AC2 and AC3) were incubated (a) at 0 °C for 7 d chilling stress (20 replications); (b) without water supply for 4 weeks drought stress (30 replications); and (c) watering with 200 mM NaCl for 4 weeks salt stress (30 replications). Leaves of the untransformed plant significantly curled and wilted under various osmotic stresses. For the survival rate measurement untransformed (WT) and T_1 transgenic plants (AC1, AC2, and AC3) were evaluated after restoring the stressed plants to normal growth conditions as described in Materials and methods. The photograph represents one set of the stress-treated plants. Numbers of surviving plants per total number of tested plants are indicated in the top of each photograph.

is clear that during drought treatment a strong expression of *CBF1* was observed when water was withheld for 3 d. The expression was switched off when the stress-treated plants were restored to normal growing conditions for 4 d. Similarly a strong expression of *CBF1* was observed during salt treatment, which was reversed when the stress-treated plants were restored to normal growth conditions (Fig. 3b). However chilling treatment triggered a noticeably low expression of *CBF1*, which was not observed when the stress-treated plants were restored to normal growing conditions (Fig. 3c).

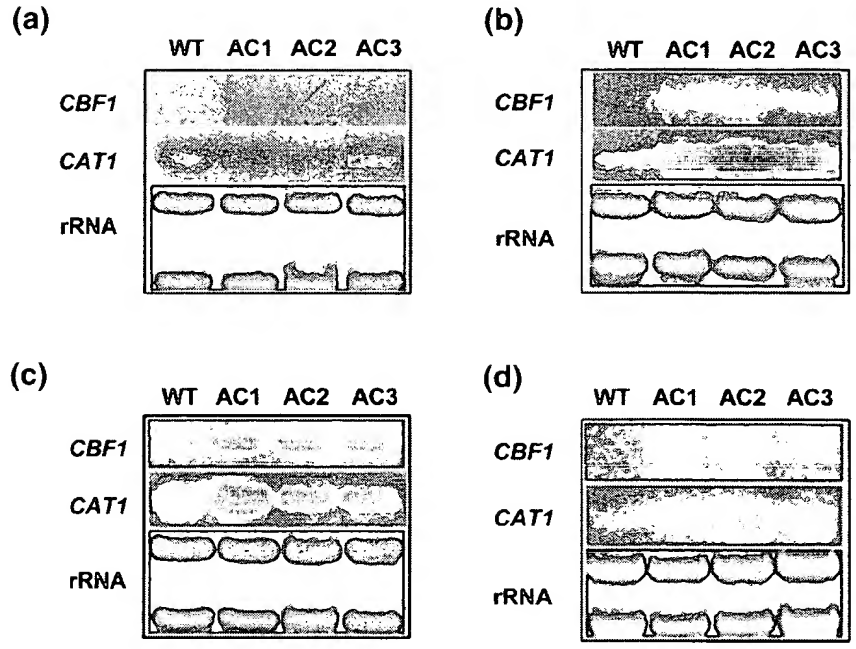

Figure 2. The heterologous ABRC1 promoter can be induced by various stress treatments to drive *CBF1* expression in transgenic tomato plants. Total RNA (10 μg) was extracted from untransformed. WT (lane 1) and transgenic ABRC1-*CBF1* T_2 plants (lanes 2–4). Probes used were ^{32}P-labelled *Arabidopsis CBF1* cDNA and tomato *CAT1*. rRNA was used as internal control. Non-stressed, untransformed and transgenic ABRC1-*CBF1* tomato served as control (a). Drought stress was induced by withholding watering for 4 weeks (b); salt stress by irrigating the plants with 200 mM NaCl for 4 weeks (c); and chilling stress by exposing plants at 0 °C for 7 d (d).

Physiological changes in ABRC1-*CBF1* transgenic plants

Plant photosynthetic efficiency, was measured by light-induced chlorophyll fluorescence (F_v/F_m) and ion leakage in order to determine the level of cellular damage following stress treatments (Fig. 4). The F_v/F_m ratio decreased in the untransformed plants under stress; however, the transgenic lines (AC1, AC2 and AC3) were less affected (Fig. 4a). Water-deficit, salt and chilling stress caused severe ion leakage in the wild-type plants, whereas the transgenic plants AC1, AC2 and AC3 were less affected as depicted in Fig. 4b. In order to determine whether any close relationship existed between stomatal movement and water imbalance, the change in leaf conductance was measured in the transgenic lines and untransformed plants. It was observed that the stomata movement increased rapidly after the start of the light period, reached a maximum level at about the sixth hour and then decreased (Fig. 5a). The stomata movement of the transgenic tomato plants decreased rapidly under various osmotic stresses after about 2 h (Fig. 5b–d). These results imply that the transgenic plants were more sensitive to osmotic stress treatment. Interestingly, ABRC1-*CBF1* expression resulted in transgenic plants retaining water, thus negating tissue damage that may have contributed to the survival of the transgenic plants when exposed to chilling and osmotic stress.

Evaluation of agronomic performance of ABRC1-*CBF1* plants

The agronomic performance of the ABRC1-*CBF1* and untransformed plants were evaluated with respect to fruit number, seed number per fruit and fresh weight of both stressed and non-stressed tomato plants. The results depicted in Table 1 clearly shows that the agronomic performance of the transgenic lines (AC1, AC2 and AC3) were similar to untransformed plants under normal growth conditions and noticeably better after stress treatments. A comparison of the fruit number and size of AC1 (ABRC1-*CBF1*) with untransformed plant and C5 (CaMV35S-*CBF1*) depicted in Fig. 6 confirms the fact that the agronomic performance of the untransformed plants is restored in the ABRC1-*CBF1* transgenic lines which, in the case of CaMV35S-*CBF1* plants could be achieved only by the application of gibberellic acid 3 (GA_3).

DISCUSSION

Plants response to chilling, drought and salt stress is the result of the responsive gene(s) and its products, thought to function in protecting cells from these stresses. In the present investigation, we were able to produce 50 transgenic tomato plants overexpressing the *Arabidopsis CBF1* driven by the stress-inducible promoter (ABRC1) of the barley *HAV22* gene. Three T_2 transgenic tomato plants (AC1, AC2 and AC3) were randomly selected for molecular characterization. It was observed that constitutive expression of ABRC1-*CBF1* in transgenic tomato plants, exhibited enhanced tolerance to chilling, drought and salt stress in comparison with untransformed plants. In our previous study we demonstrated improved tolerance of CaMV35S-*CBF1* tomato plants to chilling, drought and salt stress, but the tolerance was achieved at the expense of plant growth and yield. However in the present study the ABRC1-*CBF1* tomato plants not only exhibited

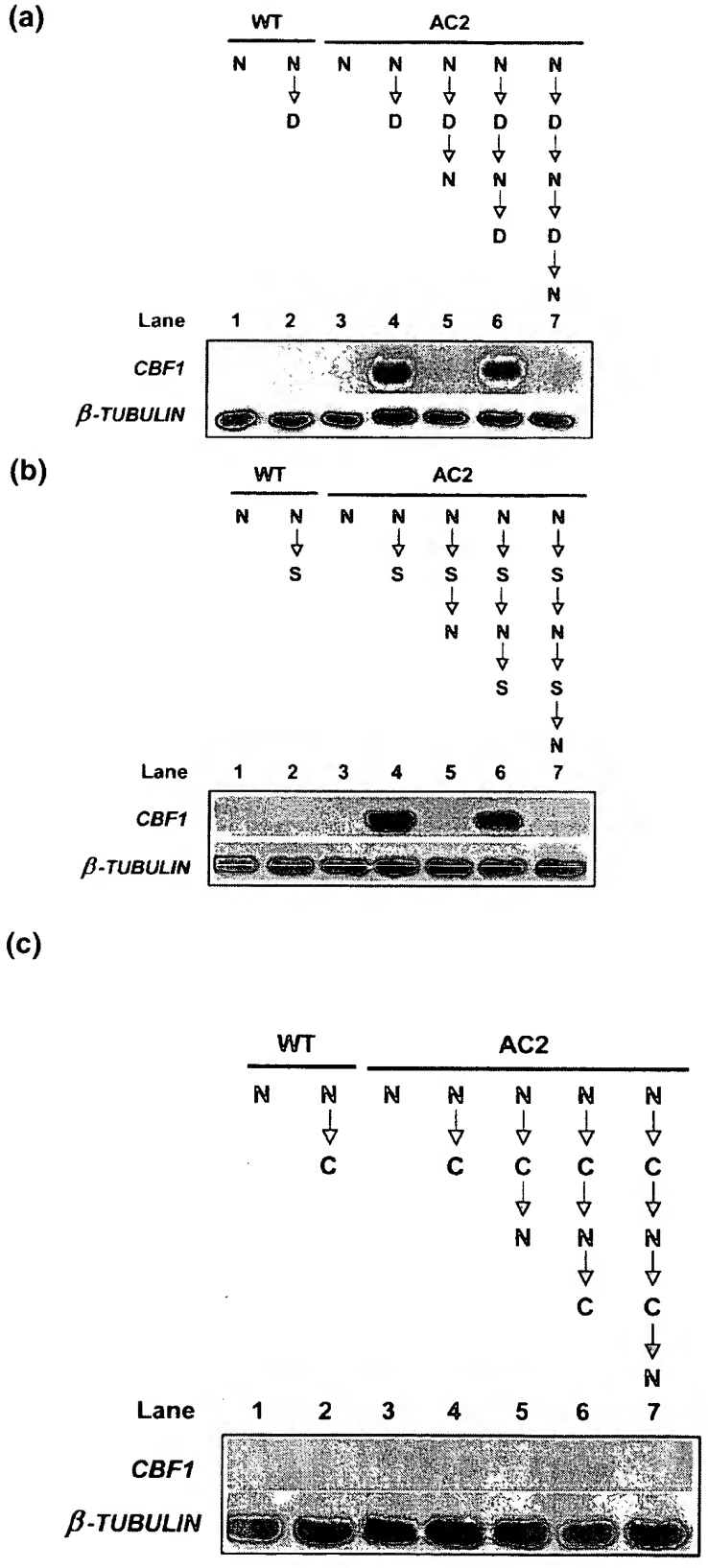

Figure 3. Reversibility of *CBF1* expression in the transgenic tomato plants. Untransformed and transgenic (AC2) tomato plants were grown under normal growth conditions. (a) Drought stress was induced by withholding water for 3 d followed by watering for 4 d. (b) Salt stress was created by growing the plants in 200 mM NaCl for 3 d followed by irrigation with water for 4 d. (c) Chilling treatment was conducted by exposing the plants to 0 °C for 2 d followed by normal growth conditions for 4 d. In all cases the treatments were repeated again. Leaves were collected at various times and total RNA isolated. Northern blots were probed with *CBF1* and *β-TUBULIN*

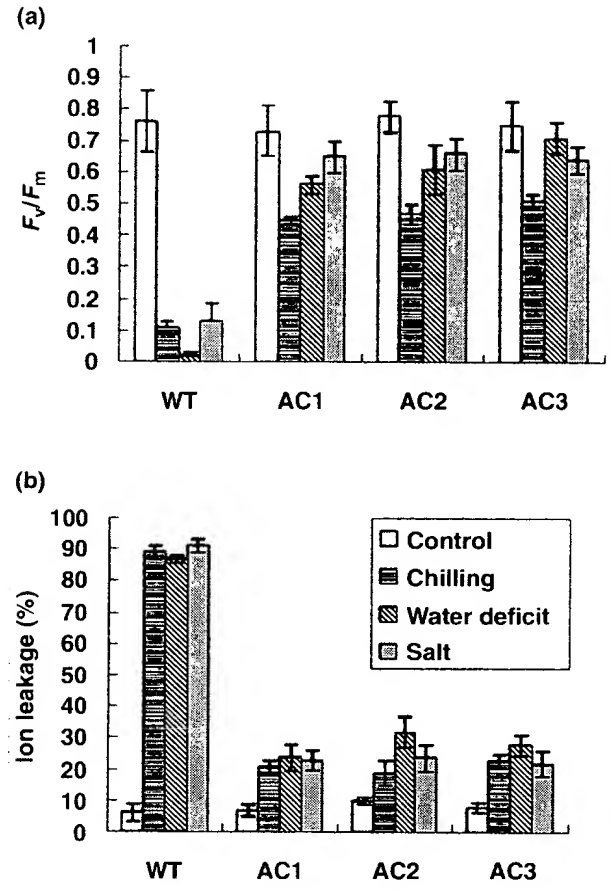

Figure 4. Improved tolerance of ABRC1-*CBF1* transgenic tomato T_2 plants to water-deficit, salt and chilling stress. Untransformed and transgenic tomato plants were subjected to chilling, drought and salt stress. F_v/F_m values (a) and ion leakage (b) were measured at the end of various osmotic stresses, respectively. Results are from average of 10 measurements (five individual plants) with less than 5% standard deviation.

enhanced tolerance to chilling, drought and salt stress, but they also maintained normal growth and yield that was equivalent to the untransformed plants under normal growth condition. It was observed that the acclimation response, growth and yield characteristics of the transgenic tomato plants were better than the untransformed plants when the former was restored to normal growth conditions after stress treatment. At the transcript level the expression of ABRC1-*CBF1* and its target gene (*CAT1*) was detected under both normal and stress conditions. We observed a low expression of the *CAT1* gene under normal condition even in the untransformed plants. However, the gene was overexpressed under various stress conditions in transgenic tomato plants in comparison with untransformed plants. Similarly a strong expression of *CBF1* was observed in the transgenic tomato plants under stress conditions in comparison with normal conditions. No signal was observed in the untransformed plants. This suggests that the scavenging effects of the catalase encoded by the *CAT1* gene might be a contributing factor for tolerance in transgenic tomato. We have made similar observations in tomato plants expressing *CBF1* driven by CaMV35S (Hsieh *et al.* 2002a). In maize (*Zea mays*), *CAT1* expression is induced by osmotic stress through two alternative signal transduction pathways, an ABA-signalling pathway and an ABA-independent pathway, mediated by two different DNA binding factors, CAT1 binding factor 1 and 2, respectively (Guan & Scandalios 2000). Tomato CAT1, maize CAT3 and rice (*Oryza sativa*) CAT-A belong to class II catalase and are expressed in vascular tissues (Dat *et al.* 2000). It has been reported that the *CAT3* gene expression and its enzymatic activities are increased during acclimation in chilling-sensitive maize. The improvement of chilling tolerance conferred by acclimation in maize is correlated with the up-regulation of the *CAT3* gene (Anderson *et al.* 1994; Prasad 1997; Dat *et al.* 2000). Transgenic tomato plants overexpressing antisense *CAT1* were more sensitive to oxidative stress and chilling injury (Kerdnaimongkol & Woodson 1999), suggesting *CAT1* plays an important role in protecting the plant from oxidative stresses. Our present results are in accordance with the above-mentioned reports and hence it can be assumed that the enhancement of stress tolerance in ABRC1-*CBF1* tomato may be partially, if not solely, due to the induction of the *CATALASE1* gene. However more evidence is needed to determine whether the heterologous *CBF1* activates *CAT1* directly or indirectly.

One interesting feature of the ABRC1-*CBF1* tomato plants is the reversibility of gene expression. The transgenic tomato plant (AC2) growing under normal conditions exhibited a low expression of *CBF1*. However when the plants were subjected to chilling, drought and salt stress a strong signal of *CBF1* was observed, which was reversed to the former level of expression when the plant was restored to normal growth conditions. This result suggests that overexpression of the gene and its down-stream gene(s) were accomplished only under stress conditions and this phenomenon could be reversed. This confirms that the transgene introduced is transcriptionally and translationally stable and the acclimatory response is effectively mediated by the presence or absence of stress.

The tomato plants overexpressing *CBF1* driven by ABRC1 promoter from barley *HVA22* gene exhibited enhanced tolerance to chilling, drought and salt stress in comparison with untransformed plants and CaMV35S-*CBF1* tomato plants (Hsieh *et al.* 2002a, b). The problem of growth retardation encountered in the latter study was overcome in the present investigation by using a stress-inducible promoter. Hence it can be hypothesized that the *HVA22* gene may be one of the target genes of the *CBF1* protein. Although this is similar to the report of Kasuga *et al.* (Kasuga *et al.* 1999), in which *Arabidopsis* plants expressing the *DREB1A* driven by the stress-inducible promoter RD29A not only exhibited enhanced tolerance to chilling, drought and salt stress but also solved the problem of growth retardation encountered in 35S-*DREB1A Arabi-*

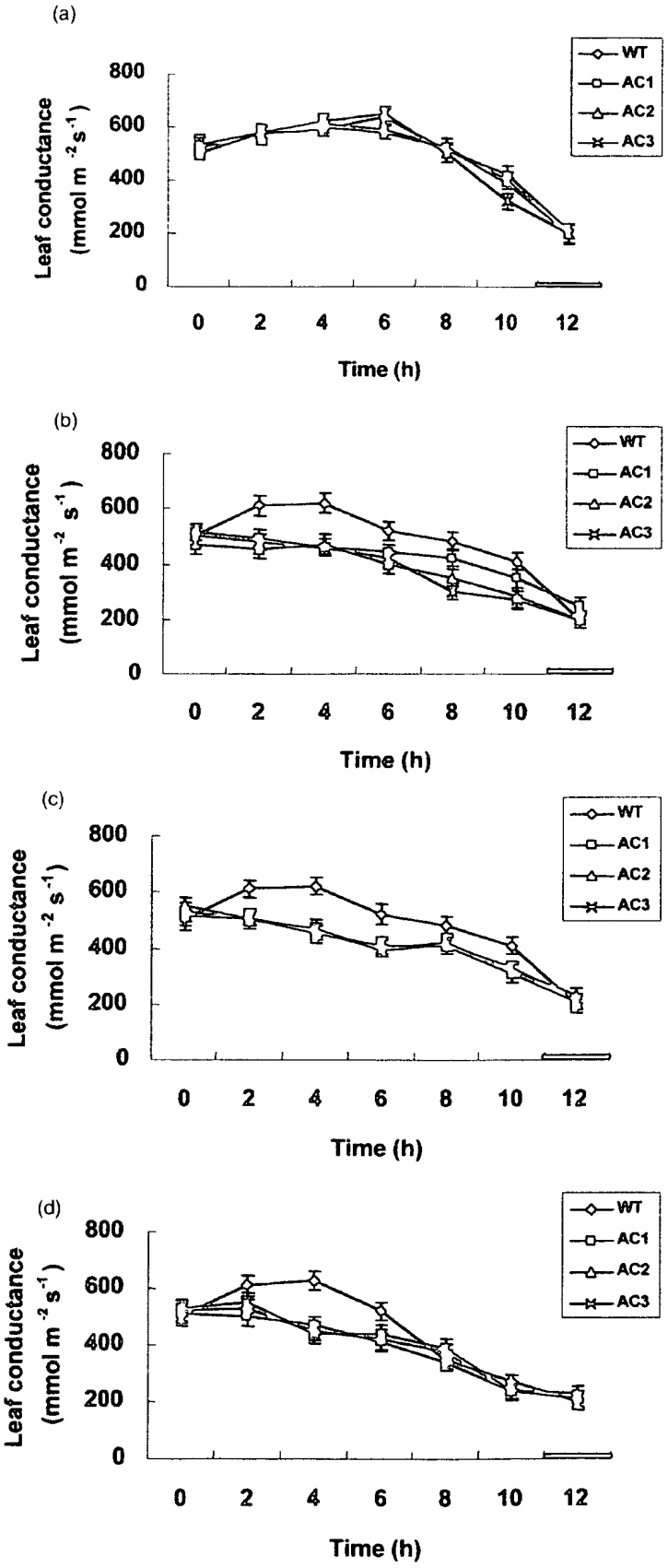

Figure 5. Transgenic tomato plants rapidly close stomata in comparison with untransformed plants under various stress conditions. Transgenic *CBF1* and untransformed plants growing under normal conditions at 24/26 °C and 16/8 h photoperiod (day/night) (control) (a); incubated at 0 °C (chilling stress) for 2 d (b); without water for 10 d (water-deficit stress) (c); or 200 mM NaCl treatment for 4 d (salt stress) (d). The period 10 to 12 h in the horizontal axis represents the dark period. Five individual plants of each line (untransformed and transgenic line) were used during each stress treatment. Results are taken from an average of 10 measurements with less than 5% standard deviation.

Table 1. The effects of various treatments on the growth characteristics of transgenic and wild-type (WT) plants

	WT	AC1	AC2	AC3
Control				
Fruit number per plant	21 ± 3.8	22 ± 4.7	20 ± 5.1	25 ± 1.6
Seed number per fruit	49 ± 7.8	47 ± 5.5	44 ± 6.0	44 ± 5.6
Fresh weight (g) per plant	133.4 ± 6.4	140.8 ± 8.7	136.4 ± 9.5	143.4 ± 13.1
Chilling				
Fruit number per plant	1 ± 1.0	14 ± 3.2	12 ± 4.1	16 ± 4.2
Seed number per fruit	2 ± 0.9	32 ± 4.9	30 ± 3.9	25 ± 1.8
Fresh weight (g) per plant	32.0 ± 6.7	102.2 ± 15.4	105.0 ± 19.3	101.0 ± 15.4
Water deficit				
Fruit number per plant	3 ± 1.6	21 ± 6.8	22 ± 2.2	31 ± 3.3
Seed number per fruit	2 ± 0.8	56 ± 5.5	54 ± 4.0	54 ± 5.7
Fresh weight (g) per plant	38.2 ± 23.6	120.8 ± 9.4	113.8 ± 8.9	115.4 ± 12.3
Salt				
Fruit number per plant	2 ± 0.4	23 ± 4.3	20 ± 4.7	25 ± 2.9
Seed number per fruit	2 ± 0.9	44 ± 7.1	49 ± 8.4	44 ± 3.7
Fresh weight (g) per plant	24.6 ± 9.3	139.4 ± 9.5	120.4 ± 9.3	119.4 ± 7.3

Each value is the mean ± SD ($n = 5$ plants). The measured plants were 3 months old. The stress treatment time is included in the growth period.

dopsis plants, in the present investigation we have proved the feasibility of this concept in a practical crop of agronomic importance. The agronomic performance of the transgenic (AC1, AC2 and AC3) and untransformed tomato plants subjected to normal growth condition after various stress treatments and non-stressed plants were evaluated with respect to fruit number per plant, seed per fruit and fresh weight. As expected the transgenic tomato plants performed better than the untransformed plants under the tested stress conditions and the yield was better than the untransformed plants growing in similar conditions. This was in contrast to the previous reports in which the 35S-*CBF1* tomato plants yielded less fruit than the wild-type plants suggesting that hyper-accumulation of *CBF1* protein or overexpression may be interfering with the gibberellic acid (GA) biosynthesis in the 35S-*CBF1* transgenic tomato plants (Hsieh *et al.* 2002a; Hsieh *et al.* 2002b). The use of a stress-inducible promoter might have overcome this limitation and thereby contributed to the normal growth and yield of the transgenic plants. Hence it can be suggested that the concept of driving a stress-responsive gene with a stress-inducible promoter can be successfully applied to agriculturally important crops.

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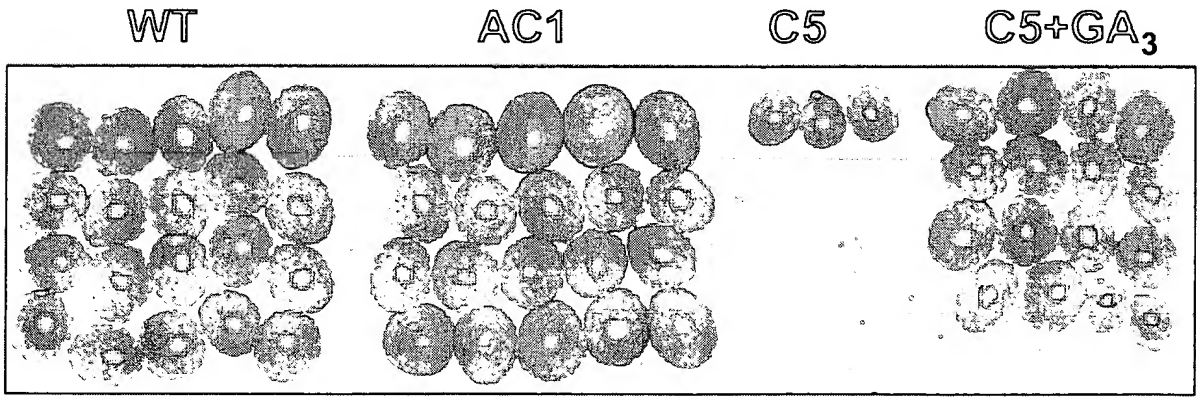

Figure 6. Improved agronomic performance of ABRC1-*CBF1* tomato plants. The yield of the transgenic tomato line (AC1) was equivalent to that of the untransformed plants. This condition in C5 plants (CaMV35S-*CBF1*) could be restored only after spraying the plants with GA_3.

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